MICROÖRGANISMS CONCERNED IN THE OXIDATION OF SULFUR IN THE SOIL

II. THIOBACILLUS THIOOXIDANS, A NEW SULFUR-OXIDIZING ORGANISM ISOLATED FROM THE SOIL[1]

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By composting sulfur, rock phosphate and soil it was found (McLean, 1918) that sulfur is rapidly oxidized to sulfuric acid; the acid acts upon the tricalcium phosphate, converting it into di- and mono-calcium salts. In the absence of a neutralizing agent or, after this agent has all been used up, the sulfuric acid formed, in the presence of an excess of sulfur, accumulates in the medium. On inoculating such composts into proper culture media, we finally succeeded in isolating a small bacterium which is active in the oxidation of the sulfur. A detailed study of the composting of sulfur, of the transformation of the tri-calcium phosphate and of the methods used in the isolation of the organism are found elsewhere (Lipman, Waksman and Joffe, 1921); only a brief review of the process of isolation is presented here.

Method of isolation. The following media were originally used for the isolation of the organism:

Medium 1:

$(NH_4)_2 SO_4$	2.00 gram
K_2HPO_4	1.00 gram
$MgSO_4$	0.50 gram
KCl	0.50 gram
$FeSO_4$	0.01 gram
Sulfur	10.00 grams
$Ca_3 (PO_4)_2$	10.00 grams
Distilled water	1000.00 cc.

Medium 2: Same as no. 1, but with 0.1 per cent glucose.

Medium 3: Same as no. 1, but in place of 10 grams only 2.5 grams $Ca_3(PO_4)_2$ per liter.

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The media were distributed in 100 cc. portions into 250 cc. Erlenmeyer flasks and sterilized in flowing steam, for 30 minutes, on three consecutive days. The flasks were then inoculated with various dilutions of the composts. Medium 2 was found to allow a growth of both a sulfur oxidizing bacterium and one or more species of fungi. By omitting the glucose from the medium, the fungi were practically eliminated.

It was found later that, by cutting down the tri-calcium phosphate in the medium to 0.25 per cent, a more rapid development of the organism took place, thus giving medium 3, which is a modification of 1 and 2.

Well advanced composts were used for inoculation. The material was diluted 10, 1000, 100,000 and 10,000,000 times with sterile water, then 1 cc. of each dilution was added to 100 cc. of the sterile medium and the flasks incubated, at 25°, for seven to fourteen days.

The flasks became turbid on the fourth or fifth day, the amount of turbidity depending upon the dilutions used, the higher dilutions developing slower than the lower ones. A pellicle or fungus mycelium was formed only in the flasks containing glucose. By transferring the cultures into fresh flasks, the same phenomenon was observed with a uniform turbidity in four to five days. By examining the culture under the microscope, it was found to contain a very minute non-motile bacterium present in abundance and accompanied by a few larger cylindrical cells which were found to be spores of a fungus occurring abundantly in the compost. The impure culture of the organisms was found to possess strong sulfur-oxidizing properties, about 200 to 300 mgm. of the sulfur being oxidized, in each flask, in fourteen days. In the presence of tri-calcium phosphate more of the sulfur is oxidized, since the acid formed is used up in converting the insoluble phosphate into soluble calcium-acid-phosphate and calcium sulfate. A further accumulation of the sulfuric acid resulted also in the formation of phosphoric acid and calcium sulfate. The medium had originally a reaction equivalent to pH 5.6 to 6.2. Following the oxidation of the sulfur, the reaction became gradually acid and, at a pH of

2.6–2.8, the reaction remained stationary till all the tri-calcium phosphate had been transformed into mono-calcium salt, after which the reaction became more acid, as shown in table 1 and figure 1.

All attempts to grow the sulfur-oxidizing organisms on solid media failed, neither agar nor silica-jelly media allowing any growth to take place.

TABLE 1

Course of reaction and accumulation of water soluble phosphates

AGE OF CULTURE	pH	PER CENT OF INSOLUBLE PHOSPHATES MADE WATER SOLUBLE*
days		
At start	5.4	
1	5.4	
2	5.3	0.9
4	4.6	5.5
6	3.5	
8	2.6	33.7
10	2.7	27.5
12	2.6	81.7
15	2.4	93.9
19	2.3	86.3
23	2.3	85.9
30	1.8	
38	1.8	86.1
68	1.7	
120	0.8	85.9

*Medium contains originally 1 per cent insoluble phosphate.

A pure culture was obtained by continued transfer in fresh flasks with high dilutions, so as to eliminate any contaminating organism, the medium being made acid at the start (pH 2.0–3.0), by the use of phosphoric acid and mono-potassium phosphate. The culture was finally obtained in a pure state. Its purity was demonstrated by repeated microscopic examinations, by the uniform growth in the liquid media and by the fact that no organism developed, when the culture was inoculated upon common bacteriological media.

On repeated transfer, the culture was found to deteriorate since it took a longer period of time to develop. It was found, necessary, in order to obtain a good growth, to use a sterile pipette instead of a loop, one or two drops being sufficient to inoculate 100 cc. By buffering the medium with suitable substances, such as phosphates, the organism would develop much more rapidly, particularly at the more acid reactions. The

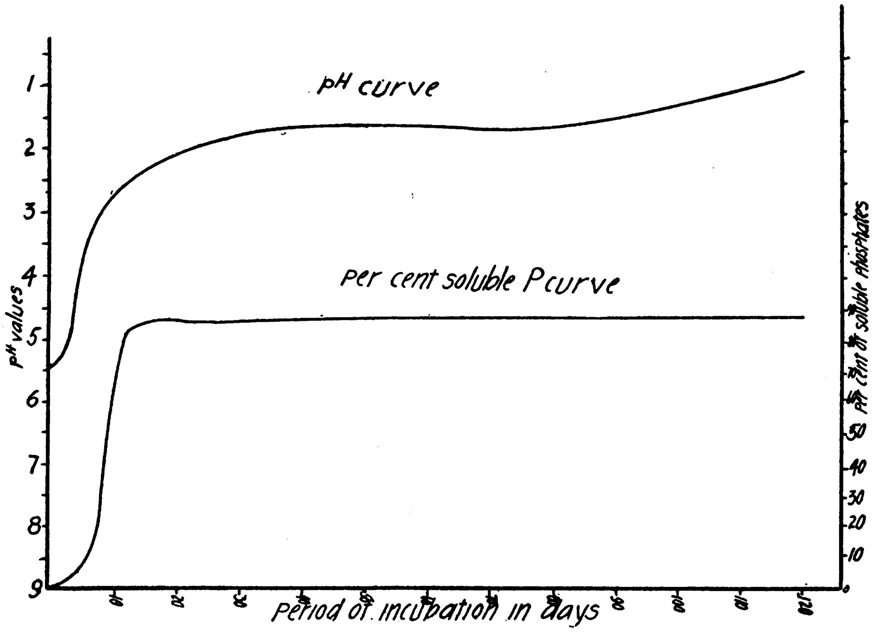

FIG. 1. COURSE OF REACTION AND ACCUMULATION OF WATER SOLUBLE PHOSPHATES IN A PURE CULTURE OF *Thiobacillus thiooxidians* N. SP.

organism was found to be morphologically similar to the two Thiobacilli described by Beijerinck, and it is, therefore, classified in that genus, under the name of *Thiobacillus thiooxidans* n. sp.

Morphology. Vegetative cells, on the synthetic media used, are short rods, with rounded ends, usually occurring singly, to some extent in pairs and rarely in triplets. The majority are less than 1 micron long and about 0.5 micron in diameter. Spore formation, absent. The majority of the cells are non-motile, although a few motile cells can also be found in young (seven days old) cultures. The organism stains well with gentian-violet and methylene blue. It is Gram-positive.

CULTURAL CHARACTERISTICS

No agar or other solid medium has been found as yet, upon which the organism would grow. It grows in liquid media with a strong uniform clouding, without any surface growth or sediment formation. It does not grow on the common organic media, although the presence of glucose or peptone in the medium is not injurious. Inorganic media containing sulfur as a source of energy are suited for its growth. In the presence of tri-calcium phosphate, the growth of the organism is accompanied by characteristic reactions: the sulfur forming originally a layer on the surface of the medium usually drops to the bottom, the sulfuric acid formed from the oxidation of the sulfur dissolves the tri-calcium phosphate giving soluble phosphate and $CaSO_4 \cdot 2H_2O$, the calcium sulfate crystallizes out in the form of radiating monoclinic crystals hanging down from the sulfur particles that are floating on the surface of the medium or protruding upward from the bottom. The reaction of the medium becomes acid as indicated by the change in the hydrogen-ion concentration. At a pH of about 2.8, the reaction becomes stationary till all the calcium-phosphate has been dissolved. In the presence of an excess of this neutralizing agent, or in the presence of rapidly dissolving alkaline carbonates, the culture is injuriously affected. Anything that will tend to change the medium to an alkaline or even a less acid reaction (except, of course, the action of the buffers), such as shaking the culture, in the presence of even smaller amounts of tri-calcium phosphate, will also tend to affect the uniform growth of the organism injuriously.

The culture can be kept alive for numerous consecutive generations on the liquid media and when not injured by an excess of alkali or acid, may be as active as a recently isolated culture.

The index No. of *Thiobacillus thiooxidans* is, according to the new Descriptive Chart of the Society of American Bacteriologists, 5332–5230–2222.

PHYSIOLOGY

Source of carbon. The organism derives all its carbon need from the CO_2 of the atmosphere. When carbon was introduced into the culture in the form of carbonates and bicarbonates, the presence of the former prevented growth due to the fact that they kept the medium alkaline, thus preventing a normal development of the organism, while the latter, if present only in small amounts, allowed a good growth to take place. But since the growth was not any better, and to some extent even worse than in the bicarbonate-free flasks, its use is superfluous. At this point, we get a clear differentiation in the metabolism of two important autotrophic organisms, the nitrifying and the sulfur-oxidizing bacteria. While the former thrives best at an alkaline reaction, the latter grow best at an acid reaction. Sodium bicarbonate is considered to be indispensable for the nitrifying bacteria; this was thought to be due to the utilization of the bicarbonate as a source of carbon, but, as recently pointed out by Meyerhof (1916), the bicarbonate merely serves the purpose of a buffer in the medium, to keep the reaction alkaline (optimum pH 8.3–9.3). In the case of the sulfur oxidizing bacterium, which has its optimum at a distinctly acid reaction (pH 3.0–4.0), the bicarbonate is not necessary since its buffering properties will tend to make the medium less acid and thus have an injurious effect, while as a source of carbon, the CO_2 from the atmosphere seems to be sufficient.

Source of energy. Sulfur is the all important source of energy for this organism. The organism is strictly autotrophic and, although glucose did not exert any injurious action, and perhaps its action was even to some extent beneficial, the amount of sulfur oxidized and acid produced were about the same in glucose and in glucose-free cultures.

In addition to sulfur, thiosulfate is also utilized, but to a much smaller extent: while, with elementary sulfur, growth appears in four to five days, under favorable conditions, as demonstrated by the turbidity and change in pH value, with thiosulfate, growth appears only in ten to twelve days and is much slower.

Hydrogen sulfide and sulfides are not utilized at all, which sharply differentiates our organism from those of Nathanson (1903), Beijerinck (1904), and Jacobsen (1914), as will be pointed out later.

Mineral requirements. Mere traces of K, Mg, Ca, Fe, in addition to phosphates, are sufficient for the growth of the organism. As a matter of fact, good growth and good sulfur oxidation were obtained by omitting, in various batches of media, each of the first four minerals, but, of course, no precaution was taken to eliminate any traces present in the distilled water or any substances that might have been dissolved out by the action of the sulfuric acid on the glass of the flask.

Source of nitrogen. Due to the very small amount of growth made by the organism, the amount of nitrogen required is very small: without introducing any nitrogen source into the medium, some growth is obtained, the nitrogen being derived either from the contamination of the other salts, the distilled water, or traces of ammonia in the atmosphere. The best sources of nitrogen are ammonium salts of inorganic acids (particularly sulfate), followed by the ammonium salts of organic acids, after which come the nitrates, asparagin and amino acids. Nitrites, in concentrations used (2 grams per liter) are toxic. Good growth is obtained with pepton, but the amount of sulfur oxidized is less than with the other sources of nitrogen.

Relation to oxygen. The organism is strictly aerobic, in view of the fact that it derives the oxygen necessary for the oxidation of sulfur to sulfuric acid from the atmosphere.

Influence of organic substances. As pointed out above, glucose does not act injuriously, neither do other organic substances, like pepton. Substances like glycerol, alcohol, mannitol and glucose seem to have a slight favorable effect in the presence of a good nitrogen source. All these substances either act like stimulants or else take part in the structural requirements of the organism.

Influence of stimulants. In addition to the pure organic substances, above mentioned, which may stimulate to some extent the growth of the organism, other substances may exert the same action. A detailed study of the influence of stimulants on the oxidation of sulfur by a pure culture of *Thiobacilllus thiooxidans* is presented in table 2.

The medium was buffered with phosphoric acid and mono-potassium-phosphate to a pH of about 3.0 It was distributed

TABLE 2

Influence of stimulants on the oxidation of sulfur

$Ca_3(PO_4)_2$ 0.25 PER CENT	STIMULANT, 0.1 PER CENT	TOTAL SULFATES IN 100 CC. OF MEDIUM MILLIGRAMS	pH	TITRATION*
		mgm.		
+	Control	241.0	3.0	1.0
+		1476.0	1.4	2.8
−		912.5	1.5	2.5
	$CaSO_4$ (0.25 per cent)	1041.0	1.4	2.7
+	Glucose	991.0	1.5	2.7
−	Glucose	1012.5	1.3	2.6
+	Mannitol	1008.0	1.6	2.5
−	Mannitol	859.0	1.5	2.5
+	Glycerol	858.2	1.6	2.5
−	Glycerol	948.5	1.4	2.6
+	Alcohol	905.0	1.6	2.5
−	Alcohol	994.0	1.4	2.8
+	Soil	1019.5	1.6	2.7
−	Soil	1226.8	1.4	2.6
+	$Al_2(SO_4)_3$	1394.0	1.4	2.9
−	$Al_2(SO_4)_3$	989.0	1.4	2.6
+	Thallium nitrate	1133.1	1.5	2.7
−	Thallium nitrate	883.5	1.5	2.5
+	$MnSO_4$	1013.4	1.6	2.5
−	$MnSO_4$	933.8	1.4	2.5

*Titration = cubic centimeter of $\frac{N}{10}$ NaOH necessary to neutralize 1 cc. of culture, with phenolphthalein as an indicator.

in 100 cc. portions in 250 cc. Erlenmeyer flasks containing 1 gram portions of powdered sulfur and the proper amounts of $Ca_3(PO_4)_2$, where present. The flasks were plugged with cotton and sterilized in flowing steam, for thirty minutes, on three consecutive days. The organic substances were sterilized separately, then added to the sterile medium. The flasks were all inoculated with one drop of the same pure culture and incubated for twelve days. At the end of that period, the pH was determined by the colorimetric method, titration was obtained from the amount, in cubic centimeters, of $\frac{N}{10}$ NaOH necessary to neutralize 1 cc. of the filtered culture using phenolphthalein as an indicator. The total sulfates were obtained by adding the amounts of soluble and insoluble sulfates: the latter were obtained by digesting the filtered residue in acidulated water and determining the sulfates in an aliquot portion.

In the presence of calcium phosphate, the largest amount of sulfur oxidized by a pure culture of the organism was obtained in medium 1, to which no stimulating agent has been added. In the absence of the tri-calcium phosphate, the amount of sulfur oxidized was appreciably less, oxidation in this case being stimulated by various substances. The most beneficial influence was exerted by the addition of a small amount of soil: this may be due to the introduction, with the soil, of a small amount of the lacking calcium salt or of some vitamine-like substance. The favorable action of the organic substances, aluminum and manganese sulfates, may be of a stimulating nature; however, this beneficial action is only very small and lies within the range of natural variability of the organism.

Influence of temperature. The optimum temperature for the activities of *Thiobacillus thiooxidans* n. sp. lies at about 28° to 30°C. Growth and sulfur oxidation are much slower at lower temperatures (18°) and at 37°C. Temperatures of 55°–60°C. are sufficient to kill the organism.

THE NATURE OF ACID FORMED AND THE INFLUENCE OF REACTION UPON THE GROWTH OF THIOBACILLUS THIOOXIDANS N. SP.

To get an insight into the true nature of the acid formed, particularly in the presence of tri-calcium phosphate, a series of tubes containing 2 cc. portions of the culture were arranged; measured quantities of $\frac{N}{1}$ NaOH were added to these, then the volume of the liquid was brought, in all tubes, to 3 cc. by the addition of distilled water. The hydrogen ion concentration of the tubes was then determined, by the colorimetric method. The results are tabulated in table 3 and graphically presented in figure 2.

TABLE 3

Titration and hydrogen-ion concentration of a 14 day old culture of Thiobacillus thiooxidans n. sp.

$\frac{N}{1}$ NaOH	pH values	
	No calcium phosphate in the original culture	0.25 per cent of tri-calcium phosphate originally present in the culture
cc.		
0	1.5	1.5
0.02	1.6	1.5
0.04	1.7	1.6
0.06	1.7	1.7
0.08	1.7	1.7
0.10	1.8	1.7
0.12	1.8	1.8
0.14	1.9	1.8
0.16	2.0	1.9
0.18	2.0	1.95
0.20	2.2	2.0
0.22	2.3	2.2
0.24	2.4	2.2
0.26	2.5	2.3
0.28	2.6	2.4
0.30	2.8	3.0
0.32	4.4	3.6
0.34	6.4	4.4
0.36	6.4	5.6
0.38	6.4	6.2
0.40	6.4	6.6
0.42	6.4	6.6
0.44	6.6	6.6
0.46	6.6	6.6
0.48	7.4	6.6
0.50	7.5	6.6
0.52	9.0	7.2
0.54	9.4	7.2
0.56		8.8
0.58		9.6

It will be observed, by glancing at the curves in figure 2, that the hydrogen-ion concentration slowly decreases, as manifested by a slow increase in the pH values, with the addition of alkali, till the pH reaches 2.8, then there is a sudden drop in the curve, to pH 6.4, when the curve again becomes slanting, followed by a second drop. This gives the buffer effect of the cultures; the buffer action is more pronounced in the presence of tri-calcium phosphate, which increases the phosphate content of the medium.

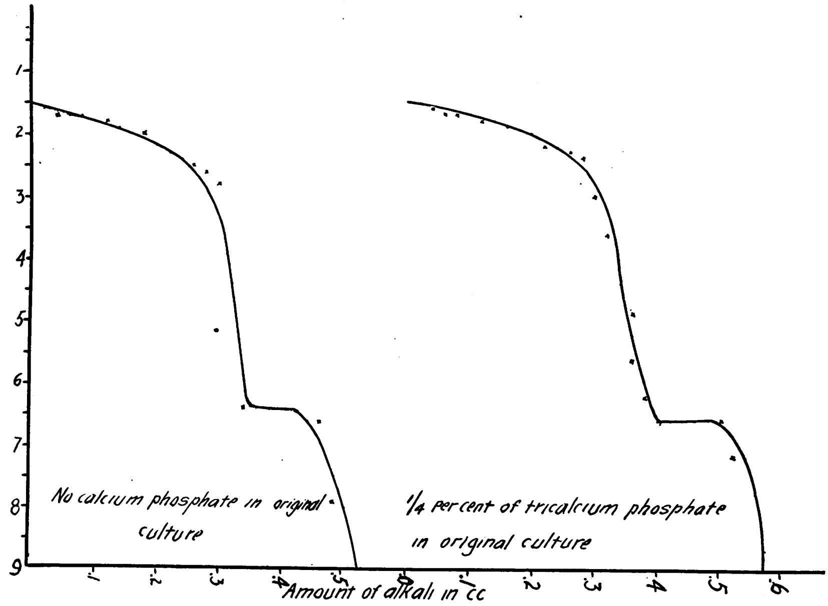

FIG. 2. TITRATION CURVES OF THE CULTURE OF *Thibacillus thiooxidans* N. SP.

The sulfur is oxidized into sulfuric acid; this acid acts upon the tri-calcium phosphate transforming it first into the di-calcium salt, then the mono-calcium salt, and finally into phosphoric acid, while the calcium is precipitated as calcium sulfate; further oxidation of sulfur results in the production of free sulfuric acid.

As to the influence of initial reaction upon growth, we find that a reaction having a hydrogen-ion concentration equivalent to a pH of 2.0–2.8 is the most favorable for the growth of the organism. Reactions more acid than 2.0 easily become injurious, although the organisms still continue to live at even as low a reaction as a hydrogen-ion concentration of pH = 0.6, while the medium titrates 0.8 normal acid (with phenolphthalein as indicator, using $\frac{N}{10}$ NaOH: the culture being grown on medium 1). Reactions ranging in pH from 4.0 to 6.0 are less favorable. Growth is slower to start, but once the reaction, through a slow oxidation of the sulfur, has reached a pH of about 3.0, the growth becomes more rapid. Reactions equivalent to pH 6.0 and above are unfavorable for growth. When a culture, at a pH 0.8 to 1.6 (these being the limits tested), is filtered free from any unoxidized sulfur, then stoppered and allowed to stand, the liquid is found to clear up, after a period of time, and the bacteria are agglutinated with the formation of flaky masses at the bottom of the containers. The rapidity of agglutination depends on the reaction of the culture, the more acid cultures agglutinating more rapidly than the less acid ones: at a pH = 0.8, agglutination took place in four to five days, while at pH = 1.5, it took more than two weeks for this phenomenon to appear. It is interesting to note that this phenomenon was never observed in the unfiltered culture, i.e., in the presence of unoxidized sulfur, even if the cultures were kept at pH 0.6 to 0.8 for a long time.

Neutralizing agents. The acid formed rapidly changes the hydrogen-ion concentration of the medium and growth almost ceases. To obviate a rapid change in reaction by the acid produced from the oxidation of the sulfur, neutralizing agents are to be used. These should be of such a nature as not to make the medium alkaline or tend to change the reaction rapidly: this eliminates, therefore, the use of carbonates and soluble oxides, like CaO. The best substances are buffers, like phosphates, but to keep the reaction above a very high acidity by means of soluble phosphates, high concentrations have to be used, which will exert an unfavorable physical effect upon the organism. Solid salts, insoluble in water which, on dissolving by the action of the acid, will give a soluble substance and an insoluble residue, are best for this purpose. $CaCO_3$ and $MgCO_3$ can be used, but these go rapidly into solution by the action of the acid, thus tending to change the reaction towards alkaline. $Ca_3 (PO_4)_2$ offers the best material for the purpose, because, on dissolving, it gives an acid salt and an insoluble residue ($CaSO \cdot 2H_2O$).

Mechanism of sulfur oxidation. The sulfur is oxidized, according to the following reaction:

$$S_2 + 30_2 + 2H_20 = 2H_2SO_4 + 282 \text{ Cal.} \qquad (1)$$

In the presence of tri-calcium phosphate:

$$Ca_3 (PO_4)_2 + 2H_2SO_4 = Ca (H_2PO_4)_2 + 2 CaSO_4 \qquad (2)$$

$$Ca (H_2PO_4)_2 + H_2SO_4 = 2 H_3PO_4 + CaSO_4. \qquad (3)$$

The energy liberated in the oxidation of sulfur is used by the organism for its activities. The acid formed interacts with the neutralizing agents of the medium, giving first mono-calcium phosphate, at a pH of about 2.8–3.0, then phosphoric acid. So that, at a condition of equilibrium, we have a mixture of phosphoric and sulfuric acids, and the calcium salts of these acids, the condition of equilibrium depending on the stage of oxidation.

Taxonomic considerations. The first paper of this series contains a study of the five groups of sulfur bacteria. The organism described in this paper, *Thiobacillus thiooxidans* n. sp., is placed in a fifth group, which includes members morphologically related to the members of the fourth group, but which are distinctly different physiologically.

Group four includes colorless sulfur bacteria which do not accumulate sulfur within their cells, but which produce an abundance of sulfur (from H_2S and thiosulfates) outside of their cells. This group of bacteria is the one closely related to the organism studied in this paper and will, therefore, be discussed in greater detail. Group four is represented by two bacteria, *Thiobacillus thioparus* (Nathanson) Beijerinck and *Thiobacillus denitrificans* Beijerinck. Group five, which is so far represented only by *Thiobacillus thiooxidans* n. sp., will include colorless sulfur-oxidizing bacteria which do not accumulate sulfur either

FIG. 3. *Thiobacillus thiooxidans*, N. SP. (× 1500). CULTURE GROWN IN INORGANIC MEDIUM

Stained with aqueous-alcoholic solution of gentian violet within or without their cells, which are very small in size (a micron or less in length and 0.5 micron in diameter) and which oxidize sulfur rapidly to sulfuric acid with a very acid reaction.

Thiobacillus thioparus was demonstrated by Nathanson in sea water and by Beijerinck in canal water. It was isolated on a medium containing sodium thiosulfate as a source of sulfur, in addition to minerals and ammonium chloride (0.01 per cent) and sodium bicarbonate as a source of carbon. In two to three days, the surface of the medium became covered with free sulfur filled with bacteria. This organism is 3 by 0.5μ, not forming any spores, is very motile and very sensitive, dying out on the plate in a week. The thiosulfates can be replaced by CaS, H_2S and elementary sulfur.

Jacobsen dissolved the sulfur in sodium sulfide and precipitated with dilute hydrochloric acid, washed and dried, then added to a medium containing 100 parts of water, 0.05 K_2HPO_4, 0.05NH_4Cl, 0.02 $MgCl_2$, 2 of $CaCO_3$ or $MgCO_3$ and a trace of $FeCl_3$ (3 parts of NaCl were used in the case of the organism isolated from sea water). The cultures were incubated at 30°C. The organism was found to form a film on the surface of the culture, with sulfur granules surrounding the cells; at the end, instead of sulfur, only a slimy bacterial mass was found to remain. Traces of hydrogen sulfide were always found. Pure cultures of the organism were obtained on agar plates, using 0.5 per cent of sodium thiosulfate and some $CaCO_3$. The carbon dioxide is obtained from carbonates, no growth being obtained and no sulfuric acid produced in the absence of carbonates. The organism is autotrophic since it does not require any organic matter for its development; it is sometimes motile and sometimes non-motile.

Thiobacillus denitrificans was isolated by Beijerinck by adding to 100 parts of canal water, 10 parts of powdered sulfur, 0.05 KNO_3, 0.02Na_2CO_3, 2$CaCO_3$, 0.02K_2HPO_4 and 0.01 part of $MgCl_2$, and incubating the medium at 30°C. The sulfur was oxidized and growth was accompanied by a reduction of the nitrate to atmospheric nitrogen. The organism was isolated on agar plates and was found to be a motile, short rod, hardly distinguishable morphologically from the *Thiobacillus thioparus*. Both organisms use carbonates and bicarbonates as sources of carbon and rapidly lose, on the plate, their ability to grow.

The following table gives the salient features of organisms belonging to groups 4 and 5.

Autotrophy. *Thiobacillus thiooxidans* belongs to the autotrophic bacteria which derive their energy from inorganic substances,

TABLE 4

Salient features of sulfur oxidizing bacteria, not accumulating sulfur within their cells

	TH. THIOPARUS (NATHANSON) BEIJERINCK	TH. DENITRIFICANS BEIJERINCK	TH. THIOOXIDANS N. SP.
Energy	H_2S, thiosulfate, sulfur	H_2S, thiosulfate, sulfur	Sulfur, thiosulfate
Size	3 by 0.5 μ	3 by 0.5 μ	1 by 0.5 μ
Accumulation of sulfur outside the cell	+++	+++	None
Pellicle formation	+	+	None
Carbon sources	Carbonates, bicarbonates	Carbonates, bicarbonates	CO_2 from atmosphere
Aerobism	Aerobic	Anaerobic	Aerobic
Growth on agar media	+	+	−
Motility	+	+	±
Acid accumulation	Active	?	Very strong, pH goes down to 0.6

and its carbon from the CO_2 of the atmosphere. This bacterium, which can derive its carbon from the CO_2 of the atmosphere, its energy from inorganic sulfur, its nitrogen from ammonium sulfate and other inorganic salts and whose mineral need is very small, was probably among the very first to start life on our planet. The sulfuric acid formed interacted with the insoluble silicates, phosphates, carbonates, etc., thus helping to break down the original rock and allowing the life of other organisms to follow. This organism or, perhaps group of organisms, together with the nitrifying bacteria may thus have formed the initial step in the organic world, manufacturing organic materials for other forms of life to follow.

SUMMARY

1. *Thiobacillus thiooxidans* n. sp. was isolated from composts of soil, sulfur and rock phosphate, by the use of inorganic media.

2. It oxidizes elementary sulfur to sulfuric acid, derives the necessary carbon from the CO_2 of the atmosphere and its nitrogen need from inorganic nitrogen salts.

3. It is responsible for the oxidation of sulfur in the soil and when soil is composted with sulfur or with sulfur and rock phosphate.

4. The sulfuric acid produced from the oxidation of sulfur by *Thiobacillus thiooxidans* n. sp. transforms tri-calcium phosphate into soluble phosphates and finally into phosphoric acid.

5. *Thiobacillus thiooxidans* n. sp. produces more acid, from oxidation of sulfur, and continues to live in a more acid medium, than any other living organism yet reported, the hydrogen-ion concentration of the medium increasing to a pH 0.6 and less.

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